SCREENING OF TEN MAIZE GENOTYPES FOR TOLERANCE TO ACIDIC SOILS USING VARIOUS METHODS

by

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DECLARATION

I, Mkafula Thembalethu Peterson, declare that the dissertation hereby submitted for the degree of Master of Science in Agriculture at the University of South Africa is entirely my work and that all reference material contained in this dissertation has been duly acknowledged. This dissertation has not been previously submitted to another university for any degree.

27 January 2020

SIGNATURE **DATE**

Mkafula Thembalethu Peterson

DEDICATION

This work is dedicated to my family

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PREFACE

This dissertation consists of five chapters. Chapter 1 gives background information and justification for conducting the study, and states objectives of the study as well as the hypotheses. A review of relevant literature and background information on methods used for various interventions are presented in chapter 2. Information on experiments that were conducted is presented in chapters 3 to 4. These two chapters are presented in paper format, complete with the introduction, objectives, hypothesis, methods and materials, results, discussion, conclusions and recommendations. There is therefore some unavoidable repetition resulting from presentation in paper format. Chapter 5 gives general conclusion and recommendations for future studies. All the references cited in the study can be found in the reference list presented after chapter 5. Appendices are listed at the end of the dissertation.

ABSTRACT

Breeding maize (*Zea mays* L.) for tolerance to acidic soils could improve maize yields. The current study aims to identify maize genotypes with tolerance to acidic soils, as well as identifying secondary traits associated with the tolerance to soil acidity. Ten maize varieties were screened for tolerance to aluminium (Al) toxicity under glasshouse, laboratory and field conditions. In the glasshouse, two soil acidity levels (limed and unlimed soil) were used and the experiment was set up in a complete randomised design (CRD) with three replications. The experiment lasted for 10 days and measurements were taken on plant height (PH), leaf area, stem diameter and dry matter. In the laboratory, a haematoxylin staining (HS) experiment was conducted to determine the response of 10 maize varieties to Al toxicity. Two Al concentrations (0 and 222 µM) were used and the experiment was set up in a completely randomized design with three replications. After 7 days, shoot length, was recorded. Five stress tolerance indices were estimated to determine the resilience of each genotype. A root growth stress tolerance index was also computed for both experimental procedures. In the field, two trials were established at two sites, namely Mbinja and Mpumaze. Limed and unlimed plots were used, and the trial was set up in a randomized complete block design with three replications. Maize kernel yield and other standard field parameters were recorded. Selection of tolerant genotypes from the field screening was also done using three indices, namely harmonic mean (HM), stress tolerance index (STI) and stress susceptibility index (SSI).

Both the glasshouse and laboratory assays identified similar genotypes of maize as being tolerant. These tolerant genotypes were Ngoyi, PANBG3492 BT, PAN 6Q408 and PHB 3442 based on the root growth stress tolerance index (RGSTI). It was therefore demonstrated that these two assays produced the same level of efficiency in identifying tolerant genotypes using this index. Based on ranking of seedling vigour index under soil acidity stress, the top three genotypes at Mpumaze were PHB32W71, PAN6616 and Sahara while at Mbinja, the top three were PAN6616, PAN6Q408 CB and PAN6P110. The genotypes PANBG3492 BT, PAN6Q408 and PHB3442 were also found to be tolerant to acidic soils at seedling stage. These genotypes are recommended for further evaluation in more sites to confirm their tolerance and yield potential under acidic soils.

The study also revealed that plant height, leaf area and stem diameter could be used for indirect selection for tolerance to Al toxicity under glasshouse conditions. The seedling vigour index was also effective in identifying tolerant genotypes under glasshouse conditions. On the other hand, shoot length stress tolerance index and the haematoxylin score were useful for indirect selection for tolerance to Al toxicity in the laboratory. In the field, it was observed that ear length, leaf area and ear diameter can be useful in identifying genotypes that are tolerant to soil acidity. They can therefore be useful as indirect selection criteria under field conditions. Additionally, the best selection indices for identifying soil acidity tolerant genotypes under field conditions were the HM and the STI. It is recommended that varieties that were identified as tolerant be further evaluated in several soil acidity hot spots to confirm their tolerance and stability of performance under field conditions.

Key words: Maize seedlings; soil acidity; stress indices; selection; tolerance.

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ABBREVIATIONS

AL	Aluminium
B	Boron
Mn	Manganese
Cu	Copper
Zn	Zinc
Fe	Iron
NaoH	Sodium hydroxide
ANOVA	Analysis of variance
DQI	Duncan quality index
ED	Ear diameter
EH	Ear height
EL	Ear length
FPRL	Final primary root length
HM	Harmonic mean
HS	Haematoxylin staining
IPRL	Initial primary root length
K/R	Kernels per rows
KY	Kernel yield
LA	Leaf area
NRG	Net root growth
NSRL	Net seminal root length
PH	Plant height
SD	Stem diameter
SHL	Shoot length
SI	Seedling vigour
SSI	Stress susceptibility index
STI	Stress tolerance index

CHAPTER 1

ORIENTATION TO THE STUDY

1.1 GENERAL INTRODUCTION

In South Africa, maize (*Zea mays* L.) is one of the main multipurpose grain crops. It is a source of food for humans and animals and has many other industrial uses (ARC, 2003). Annually, South Africa produces approximately 8 million tons of maize grain (Du Plessis, 2003). In terms of nutrition, the crop is reported to have starch, fiber, ash, sugar and proteins (Chaudhary, 1983). In the Transkei region, which is the eastern part of the Eastern Cape, families are unable to produce sufficient maize to provide for their families and have to buy maize to meet their household needs (Nompozolo, 2000; ISER, 2001). Hence, Chimonyo *et al.* (2012) reported a need to help farmers to produce enough maize using the rural farming system, which has since become the centre for programmes that seek to develop and improve rural livelihoods. However, there are numerous biotic, abiotic and socio-economic factors that affect maize production by the poor Eastern Cape farmers. Reduction in maize yields in most dryland maize growing areas occurs as a result of erratic seasonal rainfall distribution, among other factors (Du Toit *et al*., 2002). Smallholder farmers are more susceptible to climate variability and its consequences as compared to their commercial counterparts (CMMYT, 1999). According to Michael (2011), if the climate in South Africa becomes hotter and drier, maize production is projected to drop by 10 to 20% by 2050, while pests and diseases are expected to pose worsening problems. A good example is the study that was conducted in Africa, which revealed a strong statistically positive correlation between the incidence of maize streak disease and climate change (Reynaud *et al*., 2009). However, control of pests and diseases in maize fields by smallholder farmers is rarely practiced due to limited funds (Steyn, 1988).

In the Eastern Cape (EC), the soils have poor fertility coupled with the widespread occurrence of soil acidity (Beukes, 1995; Jacomina *et al*., 2009). Most of the soils surveyed by Mandiringana *et al*. (2005) from the gardens and out-fields of the former Transkei are acidic, with pH values ranging from 4.3 to 4.7%, respectively. Acidic soils are therefore a crucial challenge in the production of maize in the EC. At low pH (pH<5),

toxic aluminum ions make it difficult for roots to grow, thus reducing nutrient uptake (Sasaki *et al.,* 1996), influencing the growth of the plant and affecting the development of the entire plant (Kochian, 1995; Kidd and Proctor, 2000). The reduction of grain yield resulting from acidic soils was reported by several authors and may vary from 2.8 to 71%, the difference being based on different levels of acidity in the soil (Dewi-Hayati *et al*., 2014; Tandzi *et al*., 2015).

There are a number of options that can be used by farmers to control soil acidity. Rechcigl (1995) reported that lime increases pH and decreases H^+ and Al^{3+} ions.The solubility of other plant micronutrients such as B, Mn, Cu, Zn and Fe are reduced as the pH increases, and therefore become less toxic to plants. Liming, however, does not correct the acidity of the subsoil below the plough layer (0-20 cm), where root growth of susceptible varieties can be reduced resulting in a restriction of nutrient uptake. Furthermore, many areas where soil acidity is a significant constraint are inhabited mostly by farmers who are poor and have limited resources, and cannot afford to purchase lime.

The adoption of maize varieties that are tolerant to acidic soils constitutes a coherent and lasting subsititute to producing greater yield under low soil pH and thus preventing huge losses of grain yields often observed with maize varieties that are sensitive to acidic soils (Horst *et al*., 1997). In the end, the use of tolerant varieties is less expensive and is more sustainable and environmentally friendly. Seed companies have developed a limited range of varieties that are tolerant to low soil pH. However, it is not clear if these varieties have been widely tested, especially in the various agro-ecologies of the Eastern Cape. These varieties also do not seem to have been widely adopted by farmers. In addition, Foy *et al*. (1988) and Musunda *et al*. (2012) reported that the varieties that are found to be tolerant in one type of soil may not necessarily be tolerant in another. Therefore, there is a need for further testing of the available varieties under soil acidity stressed environments, as well as popularizing the idea of using tolerant varieties through on-farm trials.

1.2 OVERALL OBJECTIVE OF THE STUDY

- To screen ten (10) maize genotypes for tolerance to acidic soils at Mbinja and Mpumaze villages.

1.3 SPECIFIC OBJECTIVES

The specific objectives were to:

- Screen eight (08) hybrids, one (01) open pollinated variety (OPV) and one (01) landrace for tolerance to acidic soils under field conditions.
- Screen eight (08) hybrids, one (01) OPV and one (01) landrace for tolerance to acidic soils at early stages of growth in the glasshouse.
- Screen eight (08) hybrids, one (01) OPV and one (01) landrace for tolerance to aluminium toxicity at seedling stages in the laboratory.
- Identify secondary traits that are associated with tolerance to acidic soils.

1.4 HYPOTHESES TO BE TESTED

- There are no differences in the response of 10 varieties to acidic soils under field conditions.
- There are no differences in the response of 10 varieties when exposed to aluminium toxicity in the laboratory.
- There are no differences in the response of 10 varieties when exposed to acidic soils under glasshouse conditions.
- There are no traits that are highly and positively correlated with yield under soil acidity.

CHAPTER 2

LITERATURE REVIEW

2.1 MAIZE PRODUCTION IN THE EASTERN CAPE

In the Eastern Cape (EC), maize production is estimated to cover an area of 17.1 million ha (Erasmus, 1996) and is one of the most important crops (Van Averbeke, 2002). Around thirty percent of the area comprises of smallholdings on which farmers practice maize farming for home consumption and to feed their livestock. However, they are producing a very low yield of less than 1 t ha^{-1} (Musunda *et al*., 2012) due to numerous abiotic and biotic constraints that reduce maize productivity (Chimonyo *et al.,* 2012).

2.2 FACTORS CONSTRAINING MAIZE PRODUCTION IN THE EASTERN CAPE

2.2.1 Rainfall and temperature

Rainfall and temperature are dominant climatic factors affecting soil formation and stability in the EC (D'Huyvetter, 1985; Laker, 2000). For maximum production, a medium maturity grain crop requires between 500-800 mm of water (Du Plessis, 2003). The EC is characterized as having a highly heterogeneous rainfall pattern (Van Averbeke and Bennett, 2007). Rainfall received is primarily of cyclonic origin from cold fronts brought by coastal high-pressure systems, and also an orographic type in certain localities. The mean annual rainfall in the western half of the province is about 400 mm (Van Averbeke and Marais, 1991; Bothma, 2004), thus exhibiting a semi-arid climate. The eastern half, comprising the coast of the former Transkei (forming the greater part of the OR Tambo District Municipality (ORTDM)) as well as the mountainous regions of the province, has a mean annual rainfall that exceeds 1000 mm (Van Averbeke *et al.,* 2000). These parts of the province exhibit a more temperate climate.

2.2.2 Topography

The topography of the EC has been described as inconsistent by Laker (1982), while Van Averbeke *et al.* (2006) described it as being generally steep. Over half (53.3%) of the

province is covered in plateaus (areas of raised flat plains) with medium to large differences in local relief. The higher the altitude the cooler temperatures become (Shimono *et al.,* 2008). Maize grown at high altitude tends to mature later than that grown at lower altitudes (Shimono *et al.,* 2008). It is estimated that approximately one third (31.3%) of the province is mountainous but differs in local relief and agricultural potential while a small portion consists of relatively level plains (11.0%) and river valleys (4.6%) which are characterized by the occurrence of deep level lands of alluvial origin (Acocks, 1988).

According to Le Roux (2007), 56% of the EC is eroded, while Kakembo *et al.* (2007) observed gully erosion (a function of both rainfall and topography) as the most predominant form of erosion, especially on lower and middle position slopes (D'Huyvetter, 1985). Losing topsoil to erosion contributes to a loss of nitrogen, phosphorus and potassium, and a decline in potential crop yield and soil productivity (Craul, 1992). However, Mandiringani *et al.* (2005) observed better soil nutrient status on the lower positions than on the upper and middle positions of the slopes.

2.2.3 Soil depth

The EC is dominated by shallow soils owing to the dry climate in most parts of the province. External factors affecting soil formation, especially topography, prevent the accumulation of deep soils. Soil depth is an important parameter which contributes to nutrients and water storage. Shallow soils can hinder the development of the roots of a plant, preventing them from accessing nutrients (Van Averbeke and Marais 1991; Van Averbeke 2006). Maize grows best in soils with a depth of 1.5 m and deeper. Studies have shown that growing crops in deeper soils has advantages because they give greater yield than those grown in shallow soil (Van Averbeke and Marais, 1991). Van Averbeke and Marais (1991) observed severely reduced maize yield when planted in soil with the effective rooting depth of less than 1 m in the central EC even though the mean annual rainfall is about 550 mm.

2.2.4 Soil fertility and acidity

Soil pH determines nutrient availability to plants because plants grown in pH (H_2O) < 5.5 tend to show deficiencies of P, N, K^+, Ca^{2+}, Mg^{2+}, and toxicities of H^+, Al^{3+} and Mn^{2+}

(Marschner, 1991). At low pH levels, Mn^{4+} is reduced to $Mn^{2+}$and toxicity results when plants absorb an excess of Mn^{2+}. The absorbed manganese can act as a toxic agent to plants that plays a role in decreasing photosynthesis which ultimately reduces yield (Kogelmann and Sharpe, 2006). When acidic soils have high levels of iron, molybdenum deficiency occurs, especially in soils with a pH less than 5.6 (Marshner, 1991). Acidic soils can also limit root growth, as well as reducing the activity of some helpful soil microorganisms (Larson, 2008).

2.2.5 Biotic factors

In the absence of abiotic constraints in maize production, biotic factors have an equally important role in the reduction of yield. It is estimated that diseases like grey leaf spot (GLS), caused by *Cercospora zea maydis*, and various strains of rust and leaf blights, have alone resulted in a 15-75% yield reducing effect in maize grown in higher rainfall areas similar to parts of the EC. Other biotic factors such as weeds, maize stem borer, maize pollen beetles, and vertebrates, e. g. birds, have also resulted in total yield losses (De Groote *et al.,* 2004). According to Fanadzo *et al.* (2009), at post emergence stage, maize is highly susceptible to cut worm and vertebrate pest (rats and birds) damage. Fields prone to bird damage always have poor crop stands. During the post anthesis period, diseases, such as *Diplodia* cob and stem rot caused by *Stenocarpella maydis*, grey leaf spot, etc, along with maize stalk borer have been seen to reduce yields (Fanadzo *et al.,* 2009).

2.2.6 Socio-economic factors

According to Witt *et al.* (2006), the failure of many initiatives to improve maize production is due to their inability to address the socio-economic factors affecting farmers. Unlike commercial farmers who grow varieties based on market trends and yield, resource-poor farmers are bound by socio-economic and bio-physical factors (Balgah *et al.,* 2010). Issues like old age, limited input and output markets, and poverty are some of the prevailing maize production constraints (Balgah *et al.,* 2010; Bucheyeki *et al.,* 2011). Ngwadla (2002) and Baloyi (2011) reported that the majority of people involved in agriculture in rural areas are elderly, being between 51 and 89 years old, which leads to poor management, poor quality and low yields of maize. Bagamba *et al.* (2005) also reported that there is limited access to markets in the smallholder sector and farmers view

it as unprofitable to transport their produce to distant markets. This results in smallholder farmers selling their produce to neighbours and nearby villages at low prices.

Cash income in rural areas is in the form of pensions, which the farmers use to purchase farm inputs, but it is insufficient to invest in capital inputs and hiring labour (Ngwadla, 2002; Baloyi, 2011). Heidhues (1995) reported that the farmers' affordability to purchase agricultural inputs may depend solely on the financial situation of that particular household.

2.2.7 Choice of varieties

Smallholder farmers use a combination of varieties in the EC. According to studies conducted by Silwana (2000) and Sibanda (2010), the varieties include hybrids, improved open pollinated varieties (OPVs) and local landraces. According to results obtained by Silwana (2000), most farmers use traditional landraces (75%), which tend to be highly heterogeneous and give low yields. Such varieties would either be grown alone or in conjunction with certified hybrid and retained hybrid seed. However, some seed companies are providing yellow seeded varieties that are tolerant to acidic soils. It is not clear whether these varieties have been evaluated for suitability in the EC. Matiwana (2011) and Fanadzo *et al.* (2009) suggested that varieties currently in use may not be entirely adapted to the EC.

CIMMYT (1973) reported that hybrids from Mexico do not perform better than traditional varieties under low rainfall and poor fertility conditions. This could be because traditional varieties are well adapted to local conditions as compared to hybrids (Viscayno et al. 2014). The above evidence could suggest that landraces and improved OPVs might provide better yields under stress prone environments where farmers may not be able to add external inputs. However, several studies have reported that yields of hybrids exceed those of OPVs by 30-100%, with an average of 40-50% (FAO, 1997).

2.3 CAUSES OF LOW SOIL pH

The process of the development of acidic soils occurs naturally and it depends on the characteristics of the parent rock, though human interference could speed up the process (Rechcigal and Sparks, 1985; Vanbreemen *et al*., 1983). Acidic soils can also occur as a result of higher rainfall and warmer temperatures (Mandiringana *et al*., 2005). Other causes of acidification include acid precipitation (Rechcigal and Sparks, 1985; Vanbreemen *et al*., 1983), nitrification and the removal of harvestable products from fields (Australia State of the Environment Report, 2001; Matsuyama *et al*., 2005; Sirovy, 1979). There is also swift acidification related to the over use of nitrogeneous fertilisers (Guo *et al*., 2010).

2.3.1 Rainfall

Rainfall is the primary source of water for agricultural production. Ball (2010) reported that too much rainfall drains basic elements from the soil profile (calcium, magnesium, sodium and potassium) that restrain the occurrence of acidity in the soil. Mandiringana *et al.* (2005) also reported that soil acidity could be partly attributed to the higher rainfall normally observed as one moves eastward in the EC province. Thus, the Mt. Fletcher district, which generally receives the highest rainfall in the province, has more leached soils as reflected by a high proportion of soils with very low pH and Ca levels (Mandiringana *et al.,* 2005). When base cations have been leached from the soil, the percentage of aluminium and hydrogen is increased relative to other cations, which lowers the pH (Donald, 2003).

2.3.2 Fertilization

Balanced fertility management is necessary for higher productivity. Maize needs sufficient nutrients, particularly nitrogen, phosphorus and potassium, to obtain maximum yield production (Onasanya *et al*., 2009). However, Ball (2010) reported that ammonium fertilizers react in the soil in a process called nitrification to form nitrates. Hydrogen ions are released during this process, resulting in soil acidification.

2.3.3 Removal of residues after harvesting

Plants obtain nutrients from two natural sources, that is, crop residues and soil minerals (FAO, 1997). Human-induced nutrient depletion can be attributed to insufficient inputs for the replacement of nutrients taken from the soil by plants during harvesting and / or the removal of crop residues (Kumar and Goh, 2000). Harvesting of crops influences soil acidity development as the crops absorb the lime-like elements (calcium, magnesium, and potassium) that prevent soil acidity (Ball, 2010). Crop residues left on the soil surface could prevent the erosion of the soil and enhance the chemical and physical properties of soils by providing a substrate for soil microbes. However, farmers are unable to leave crop residues on their fields for soil protection because they usually remove them during the dry season as supplementary feeding of their livestock (O'Niell, 1999). If residues are returned to the soil, soil organic matter (SOM) and nutrient levels will improve while the soil pH will tend to rise.

2.4 STRATEGIES FOR MANAGEMENT OF ACIDIC SOILS

2.4.1 Use of lime

When lime is spread and incorporated into the topsoil, existing top soil acidity will be neutralised, while regular maintenance applications of lime will help prevent re-acidification. Liming, by increasing pH, eliminates nutrient deficiencies, alleviates toxicity effects and stimulates microbial activity (ARC, 1995; Rechcigal, 1995). Pollution and toxicity hazards are effectively curbed if soil pH is raised above 5.0-5.5. By increasing the pH and supplying Ca, the population and activity of beneficial soil fauna, such as earthworms, can also be increased. On some soils, this can be important because earthworms can increase the rate of organic matter breakdown and improve the physical properties of the soil (ARC, 1995). Liming ameliorates the topsoil but does not eliminate acidity in the subsoil, where it brings a huge problem to developing roots (Toma *et al*., 1999; Sierr *et al*., 2006).

2.4.2 Use of crop residues

Crop residues are also used to raise soil pH. Many of the beneficial effects of the application of crop residues to acidic soil can be attributed to their effect on increasing

soil pH and decreasing phytotoxic Al in soluble form (Noble *et al*., 1996). The addition of crop residues to soils can result in an increase in soil pH (Hoyt and Turner, 1975; Hue, 1992; & Noble *et al*., 1996). For example, Hoyt and Turner (1975), recorded an increase in soil pH of about 0.5 of a pH unit when lucerne meal was added to an acid soil. Although the pH declined again after about 20 days' incubation, it still remained above the initial soil pH value. Several other researchers have also observed an initial rise in soil pH due to the addition of crop residues, followed by a decline in pH (Hue, 1992; Wong *et al*., 1998). Since the addition of crop residues to soil often results in an increase in soil pH, a decrease in the concentration of exchangeable Al would also be expected to occur. However, smallholder farmers do not have the option of leaving crop residues on the ground for soil protection because they are required for fodder, as we have already noted.

2.4.3 Use of organic matter

The crucial key role that soil organic matter (SOM) plays includes the provision of nutrients and amending physical properties of the soil to improve crop growth (Miller *et al*., 2009; Obour *et al,* 2010; Busscher *et al.,* 2010). Whalen *et al.* (2000) observed the reduction of soil pH after the soil was amended with cattle manure. The EC farmers apply kraal manure to address the problem of soil infertility in their maize fields (Van Averbeke and De Lange, 1995). However, smallhoder farmers' manure application rate of 0.5 tons per hectare are extremely low (Bembridge, 1984), compared with the general recommendation of 10 tons per hectare (Van Averbeke and Yoganathan, 2003). The problem of acidic soils therefore persists despite the limited use of organic matter in the form of different types of manure.

2.4.4 Use of tolerant varieties

In recent years, it has been shown that in common with several other crop species, maize genotypes frequently display appreciable differences in their ability to absorb and utilize mineral elements in acidic soils (Farina, 1982). ARC (1995) reported that maize can tolerate pH ranges of 4.5-4.8 (KCL), with acid saturation levels of 5-10%. However, maize is sensitive to pH ranges of 4.3-4.6 (KCL), with acid saturation levels of between 20-30%, which may result in poor maize yields. The use of soil acidity tolerant maize varieties institutes a coherent and lasting alternative for the production of greater yields under low soil pH and the prevention of huge losses of grain yields with maize varieties that are

sensitive to acidic soils (Horst *et al*., 1997). In the end, the use of tolerant varieties is cheaper, more sustainable and more environmentally friendly.

A number of varieties that are tolerant to acidic soils have been developed in South Africa. For example, Pannar seed company developed a yellow maize variety (PAN 6966-medium maturity), which is tolerant to acidic soils making it ideal for areas with high rainfall (Pannar, 2018). However, the problem we still have is that there are few such varieties, and their resistance may not be upheld in all agro-ecologies of the EC. Foy *et al.* (1988) also reported that plant species vary widely in their ability to grow and yield on acidic soils and in different agro-ecologies. This variation in tolerance necessitates breeders to develop more acidic soil tolerant varieties that are better suited for use in most agro-ecologies of the EC, giving high yields to farmers. When such tolerant varieties have been developed, they should be tested in as many agro-ecologies as possible, to identify those areas for which they are well adapted.

2.5 METHODS USED TO SCREEN VARIETIES FOR TOLERANCE TO LOW SOIL pH

The evaluation of maize genotypes for tolerance to acidic soils in general and for Al toxicity have been done using different screening methods. Such methods include cell and tissue culture (Conner and Meredith, 1985), nutrient solution culture (Baier *et al.* 1995), soil bioassays (Stolen and Andersen, 1978; Ring *et al.,* 1993) and field evaluation (Johnson *et al.,* 1997). Laboratory and glasshouse screening for tolerance to soil acidity is important in that it enables identification of genotypes with tolerance at seedling and other early growth stages. A very large number of genotypes can also be screened in a short time using these two methods, and this can be very useful in breeding programmes where large numbers of entries may have to be assessed. Screening of maize genotypes using the field evaluation method is important because yield data will be obtained. A tolerant variety should be able to give high yields under soil acidity. Foy *et al*. (1988) and Musunda *et al.* (2012) indicated that the varieties that are found to be tolerant in one type of soil may not necessarily be tolerant in another. This calls for screening of varieties for tolerance in numerous environments. The use of different methods to screen for tolerance could assist in identifying genotypes with tolerance at different stages of growth. This could contribute to the development of whole crop tolerance to soil acidity.

2.6 SECONDARY TRAITS ASSOCIATED WITH TOLERANCE TO SOIL ACIDITY

During the breeding of maize varieties for tolerance to low soil pH, the identification of secondary traits associated with tolerance is crucial due to their correlation with yield (Tandzi *et al*., 2018). In addition, these traits could be helpful for plant genotypic characterization in response to low soil pH stress. Genetic improvement for aluminium tolerance can be simplified in the presence of genetic variation and traits that are highly correlated to grain yield under AL toxicity (Chanda *et al*., 2015). Root length assessment is one of the bases for evaluation of aluminium tolerance. Paula (2011) reported that seminal root length in the laboratory was highly correlated with field grain yield. Tandzi *et al*. (2018) also reported a high and positive correlation between leaf area and field yield. Foy *et al*. (1993) reported high and positive correlations between root growth and plant height with yield. Tandzi *et al*. (2018) also reported that root growth and plant height were found to predict field performance under Al toxic soils. This indicates that such traits could be useful in identifying genotypes that are tolerant to high acid saturation. All these analyses propose that different traits could be used for Al tolerance screening and therefore each breeding programme should find the optimal traits to use.

CHAPTER 3

RESPONSE OF MAIZE (*ZEA MAYS* L.) GENOTYPES TO SOIL ACIDITY IN CONTROLLED ENVIRONMENTS IN THE EASTERN CAPE, SOUTH AFRICA

ABSTRACT

Breeding maize for tolerance to acidic soils could improve maize yields. The current study aimed to identify maize genotypes with tolerance to highly acidic soils as well as finding secondary traits associated with tolerance to soil acidity at the seedling stage. Ten maize varieties were screened for tolerance to soil acidity under glasshouse conditions as well as in the laboratory. In the glasshouse, two soil acidity levels (limed and unlimed soil) were used and the experiment was set up in a randomized complete block design (RCBD) with three replications. The experiment lasted for 10 days and measurements were taken on plant height (PH), leaf area, stem diameter and dry matter. In the laboratory, a haematoxylin staining (HS) experiment was conducted to determine the response of the 10 maize varieties to aluminium (Al) toxicity. Two Al concentrations (0 and 222 µM) were used and the experiment was set up in a CRD with three replications. After 7 days, shoot length, was recorded. Five stress tolerance indices were estimated to determine the resilience of each genotype. A root growth stress tolerance index was also computed in both experimental procedures. The glasshouse and laboratory assays identified similar tolerant genotypes of maize as being tolerant. These tolerant genotypes were Ngoyi, BG3492 BT, PAN 6Q408 and PHB 3442, based on the root growth stress tolerance index (RGSTI). It was therefore demonstrated that these two assays produced the same level of efficiency in identifying tolerant genotypes using the RGSTI. Plant height, leaf area and stem diameter could be used for indirect selection for tolerance to Al toxicity under glasshouse conditions. On the other hand, shoot length stress tolerance index and the haematoxylin score were useful for indirect selection for tolerance to Al toxicity in the laboratory.

Key words: Maize; seedling; tolerance; Al toxicity; selection.

3.1 INTRODUCTION

In the Eastern Cape, South Africa, most smallholder farmers grow maize, which is a staple cereal food crop (Mandiringana *et al*., 2005). The production of maize in the smallholder sector is fraught with numerous biotic and abiotic constraints that result in low yields. Hence, maize yields range from less than 1 t/ha in rain-fed production systems to less than 3 t/ha under irrigation (Fanadzo *et al*., 2010). This makes it hard to attain provincial targets of self-sufficiency to feed a growing rural population of approximately 6.5 million (Statistics South Africa, 2018).

Abiotic maize production constraints include low soil pH or soil acidity. Acidic soils are a major problem worldwide in plant production. Soil acidity is generally characterized by low pH, and toxic levels of aluminium (Al), iron (Fe) and manganese (Mn). It is also associated with deficiencies of calcium (Ca), magnesium (Mg) and phosphorus (P), which cause low soil fertility (Onwuka, 2009). Maize yield losses due to soil acidity have been found to range from 2.8 to 71% (Larson, 2008 and Tandzi *et al*., 2015).

There are several management practices to ameliorate low soil pH (Tandzi *et al*., 2018). The *et al.* (2006) stated that lime improves maize yields when it is applied to acidic soils. However, soil liming is not always affordable for small scale farmers and is not environmentally friendly (Tandzi *et al.*, 2015). The use of acidic soil tolerant maize varieties institutes a coherent and lasting alternative for the production of greater yields under low soil pH and the prevention of huge losses of grain yields often observed with maize varieties that are sensitive to acidic soils (Horst *et al.*, 1997). In the long run, the use of tolerant varieties is affordable, sustainable and more environmentally friendly.

Different evaluation procedures have been used to assess Al tolerance of different crop genotypes. Such methods include tissue culture (Conner and Meredith, 1985), nutrient solution culture (Baier *et al.*, 1995), soil bioassays (Stolen and Anderson, 1978; Ring *et al*., 1993) and field evaluation (Johnson *et al*., 1997; Tandzi *et al.*, 2015). Screening for tolerance under nutrient solution in the laboratory and in the glasshouse, has been found useful for improving tolerance to soil acidity. Both techniques are particularly relevant for the preliminary evaluation of tolerance to soil acidity at the early stages of plant growth. Early crop growth stages are important as they ultimately determine the final

plant stand, and therefore yield per hectare. It is therefore important to identify maize varieties with tolerance to soil acidity during the vulnerable seedling growth stages.

Different indices have been used to classify tolerant genotypes under stress conditions. Different screening indices reflecting stress tolerance have been suggested based on relative grain yield from stress and non-stress conditions (Francisco *et al*., 2010). Adebisi *et al*. (2014) reported that a crucial quality trait that needs to be evaluated to provide germination and viability tests to gain insight into the performance of a seed lot in the field is seed vigour index (SVI). The Dickson quality index (DQI) was originally designed by Dickson *et al.* (1960) to assess the quality of seedlings. Rosielle and Hamblim (1981) defined mean productivity (MP) as the average yield of a genotype under constructive stress and optimal conditions. Fernandez (1992) defined a new index, the stress tolerance index (STI), which can be used to find genotypes with higher yields under both stress and non-stress conditions. The objectives of this study were: (i) to identify maize genotypes with tolerance to soil acidity and, (ii) to identify secondary traits associated with tolerance to soil acidity at the seedling stage.

3.2 MATERIALS AND METHODS

Maize genotypes were evaluated using two screening methods, namely the pot culture technique in the glasshouse, and the haematoxylin assay in the laboratory.

3.2.1 Plant material

Farmers from Mbinja and Mpumaze villages of Mhlontlo local Municipality use yellow maize varieties for human consumption and for livestock feeding, therefore, ten yellow maize varieties consisting of eight hybrids, one open pollinated variety (OPV) and one local landrace were used in the experiments (Table 3.1).

Table 3.1 Maize varieties that were evaluated for tolerance to soil acidity in the glasshouse and laboratory

Name	Type	Origin	Grain colour
PAN6966	Hybrid	PANNAR	Yellow
PAN6616	Hybrid	PANNAR	Yellow
PAN6Q408 CB	Hybrid	PANNAR	Yellow
PANBG3492 BT	Hybrid	PANNAR	Yellow
PAN6P110	Hybrid	PANNAR	Yellow
PHB33H56	Hybrid	PIONEER	Yellow
PHB32W71	Hybrid	PIONEER	Yellow
PHB3442	Hybrid	PIONEER	Yellow
SAHARA	OPV	DELTA	Yellow
NGOYI	Local landrace	TSOLO FARMERS	Yellow

Pot culture assay

The experiment was conducted at the University of Fort Hare research farm in April 2015. It was set up in soil-filled pots in a glasshouse with uncontrolled temperature and humidity. The research farm is in Alice (32º 46’ S and 26º 50’N), at 508 m above sea level, in the central part of the Eastern Cape, South Africa. The soils used in the experiment were collected from two sites (Mbinja and Mpumaze) regarded as acidic soil hot spots in farmers’ fields in Mhlontlo Local Municipality.

The experiment was laid out in a complete randomised design (CRD) with three replicates. Each plot had 5 polyvinyl chloride (PVC) pipes that were irrigated regularly so as to avoid moisture stress. The PVC pipes were 11 cm in diameter and 25 cm in length. Three maize seeds were planted per pipe and thinning was subsequently done to leave one seedling per pipe.

3.3 HAEMATOXYLIN ASSAY

The haematoxylin assay was conducted at the university of Fort Hare, in the genetics and botany research laboratories. Twenty-five seeds of each variety were washed with distilled water and then sterilized in a 1% solution of sodium hypochlorite prepared by mixing sodium hypochlorite and distilled water in a ratio of 1 / 100 (Gudu *etal*., 2001). The seeds were agitated for 5 minutes and then rinsed twice with distilled water. The sterilized seeds were placed in Petri dishes lined with absorbent paper and moistened with distilled water (Gudu *et al*., 2001). These were placed in the incubator which was set at 26^{o}C (Ligeyo *et al*., 2001; Magnavaca *et al*., 1987) for 5 days. On the fifth day, initial root growth measurements and visual observations were conducted. Only seedlings that had uniform germination were allowed to grow to seven days in 'no Al' and 'Al' containing nutrient solution cultures in a growth chamber (Giaveno *et al*., 2000). The growth chamber was set to a constant temperature of 26 ^{o}C, relative humidity (RH) at 75%, and 16 hours of light and 8 hours of darkness. The nutrient solution was prepared following the method of Magnavaca *et al.* (1987). The laboratory experiment was set up in a CRD with three replications and two aluminium treatments (0 and 222µM) added in the form of AlK $(SO_4)2.16H_2O$. Five litre solutions were prepared and used for each level of aluminium and pH was adjusted at 4.0 daily using 0.1M HCl and NaOH (Gudu *et al*., 2001).

3.4 DATA COLLECTED

Seedlings were cut at the initial whorl of the adventitious crown roots and the following data were collected from the glasshouse: plant height (cm), leaf area (cm^2), stem diameter (mm), and dry matter (g). Data collected in the laboratory consisted of shoot length, which was measured in centimeters. Visual scores for root staining intensity were made on a scale of 1-5 using a microscope, as follows: non-stained roots were classified as very tolerant (1), faintly stained roots as tolerant (2), moderately stained roots as moderately tolerant (3), well stained roots as sensitive (4) and those with deeply stained roots as very sensitive (5) (Ouma *et al*.,2013).

Stress tolerance indices involving root growth and shoot length were computed for both experiments. Stress tolerance indices were calculated as follows:

- The **seed vigour index (SVI)** was calculated according to the formula by Abdul-Baki and Anderson (1973): Seed Vigour Index = shoot length × germination percentage.
- The **Dickson quality index** (DQI) was calculated according to the formula by Dickson *et al*. (1960): Seedling dry weight/ [(height/diameter) + (Shoot dry weight/root dry weight)].

  All the stress tolerance index (STI) below were calculated according to Wilkins (1957).
- **Leaf area stress tolerance index** (LASTI) Leaf area of stressed plants/ Leaf area of control plants X 100.
- **Shoot length stress tolerance index (SLSTI)** = Shoot length of stressed plants/Shoot length of control plants X 100.
- **Root growth stress tolerance index (RGSTI)** = Root growth of stressed plants/Root growth of control plants X 100.
- **Initial primary root length stress tolerance index (IPRLSTI)** = Initial primary root length of stressed plants/ Initial primary root length of control plants X 100.
- **Final primary root length stress tolerance index (FPRLSTI)** = Final primary root length of stressed plants/ Final primary root length of control plants X 100.
- **Lateral seminal root length (LSRLSTI)** = Lateral seminal root length of stressed plants/ Lateral seminal root length of control plants X 100.
- **Lateral root number stress tolerance index (LRNSTI)** = Lateral root number of stressed plants/ Lateral root number of control plants X 100.

3.5 DATA ANALYSIS

SAS version 9.2 was used for data analysis. Linear analysis of variance (ANOVA) was performed for all the traits collected per genotype (G) and mean separation was done using the Tukey's test at the 5% level of probability. Pearson's correlation analysis was performed for the traits collected under unlimed and limed conditions.

3.6 RESULTS

3.6.1 Haematoxylin assay

3.6.1.1 Variance for parameters estimated in the laboratory

Significant differences were observed between genotypes for shoot length under 222 µM Al and for the majority of indices that were estimated. Non-significant differences were observed for shoot length under control conditions (without Al) and for the shoot stress tolerance index (SLSTI) (Table 3.2). Under Al stress, the haematoxylin staining estimates showed highly significant differences among genotypes. The significance of values observed among genotypes for some parameters confirmed that the concentration of Al used in the experiment was sufficient to discriminate evaluated varieties based on their level of tolerance.

Table 3.2 Mean squares for shoot length of maize varieties and estimates of evaluated and associated tolerance indices

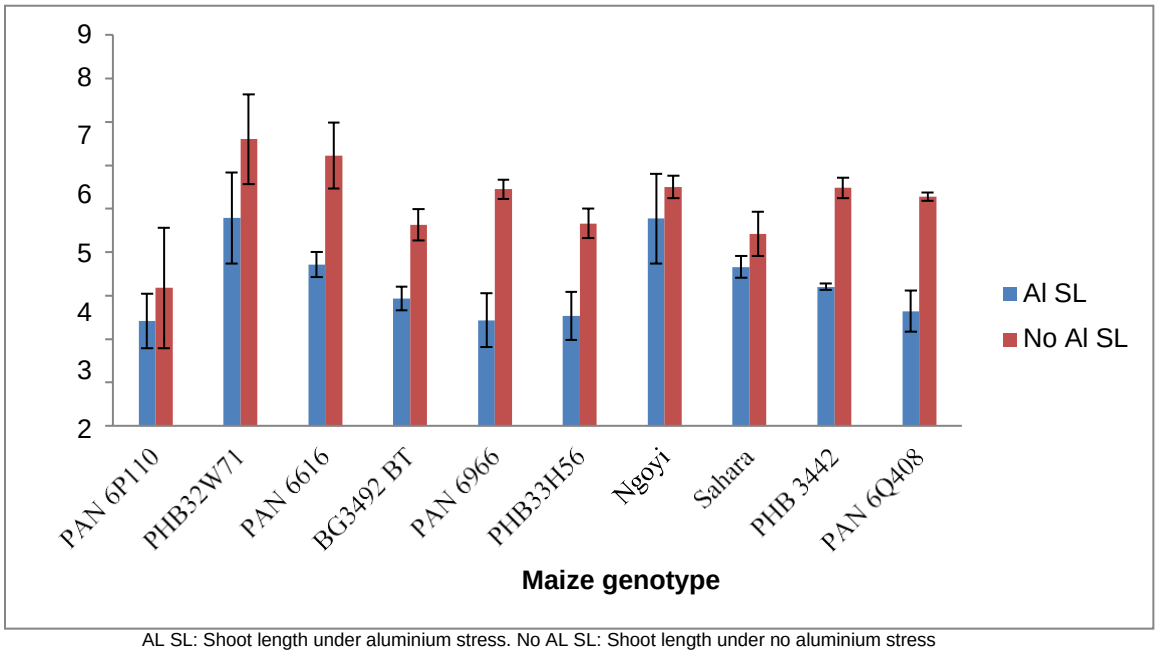
Source	DF	Al SL	No Al SL	IPRLSTI	FPRLSTI	NRGSTI	SLSTI	LSRLSTI	LRNSTI	Al HS	Al HS
Rep	2	1.8*	0.8NS	0.03NS	0.08NS	0.15*	0.06NS	0.008NS	0.06NS	2.8*	0.23NS
Var	9	2.4**	2.8NS	0.32*	0.30*	0.38***	0.07NS	0.1**	0.42**	1.14NS	3.02***
Error	18	0.5	1.4	0.09	0.1	0.04	0.06	0.02	0.09	0.69	0.12

Al Shoot length: Al SL; No Al LSR L: No Al SL; initial primary root length stress tolerance index: IPRLSTI; Final primary root length stress tolerance index: FPRLSTI; net root growth stress tolerance index: NRGSTI; shoot length stress tolerance index: SLSTI; lateral seminal root length stress tolerance index: LSRLSTI; lateral root number stress tolerance index: LRNSTI; Al HS: haematoxylin staining under no Al medium; Al HS: haematoxylin staining in Al medium; replication: rep; variety: var; ***: significant at 0.1%; **: significant at 1%; *: significant at 5%; ns: non-significant.

3.6.1.2 Mean performance of genotypes for various variables and associated indices

3.6.1.3 Shoot length of varieties evaluated

The shoot length of genotypes varied significantly from 2.42 cm (PAN6P110) to 4.79 cm (PHB32W71) under stressed conditions and from 3.18 cm (PAN6P110) to 6.60 cm (PHB32W71) under non-stressed conditions (Figure 3.1). Three genotypes also expressed relatively high performance of shoot length namely: Ngoyi (4.77cm), PAN 6616 (3.71cm) and Sahara (3.66 cm).



AL SL: Shoot length under aluminium stress. No AL SL: Shoot length under no aluminium stress

Figure 3.1 Shoot length of genotypes evaluated under 222 µM and 0 µM Al concentration (p=0.05)

3.6.1.4 Haematoxylin staining estimates under Al stress

Five genotypes showed low haematoxylin staining values (Al HS) under Al toxicity, though there were no significant differences observed between the varieties. Varieties with lower Al HS were BG3492, PHB33H56, PAN6Q408, PHB3442 and PAN6966 (Figure 3.2). These genotypes expressed good tolerance under Al toxicity.

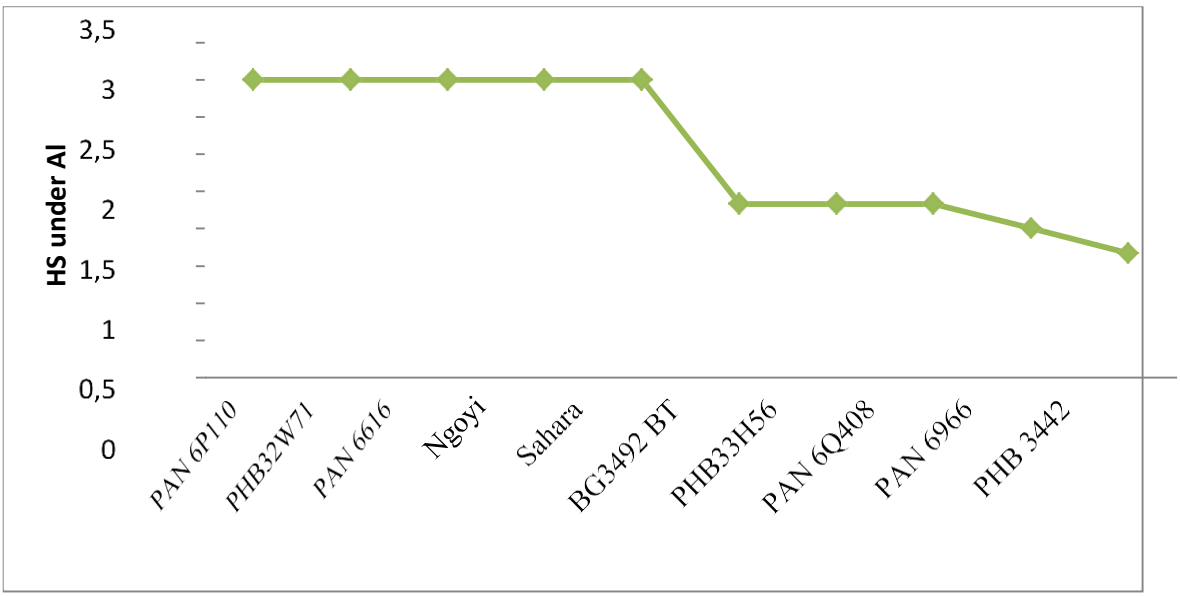


Figure 3.2 Haematoxylin staining estimates of maize varieties that were evaluated

3.6.1.5 Mean performance of genotypes based on estimated tolerance indices

The genotypes were ranked based on the stress tolerance indices (Table 3.3). The ranks of the genotypes were different from one stress tolerance index to another with a few coincidences. Based on the IPRLSTI, the top five varieties were PAN 6616, Sahara, PAN 6966, PHB33H56 and PHB 3442. The top five varieties for FPRLSTI were PHB32W71, Sahara, PHB 3442, BG3492 BT and PHB33H56. The top five varieties for NRGSTI were PHB33H56, Ngoyi, PAN 6Q408, PHB32W71 and Sahara. The top five varieties for LSRLSTI were PHB32W71, Ngoyi, PAN 6Q408, PHB 3442 and Sahara. Finally, the top five varieties for LRNSTI were PAN 6616, Ngoyi, PHB33H56, PAN 6966 and Sahara.

Table 3.3 Mean performance of genotypes based on estimated tolerance indices

Variety	IPRLSTI*	FPRLSTI	NRGSTI	SLSTI	LSRLSTI	LRNSTI
PAN 6P110	1.32 (7)	1.26 (7)	0.39 (6)	0.82 (6)	0.92 (6)	1.08 (1)
PHB32W71	0.73 (8)	0.59 (1)	0.29 (7)	0.74 (1)	0.58 (9)	0.78 (2)
PAN 6616	0.84 (1)	0.56 (8)	0.04 (10)	0.62 (8)	0.53 (1)	0.80 (3)
BG3492 BT	0.83 (6)	0.77 (4)	1.00 (2)	0.68 (7)	0.53 (8)	0.82 (6)
PAN 6966	0.73 (3)	0.68 (6)	0.24 (8)	0.45 (9)	0.56 (4)	0.66 (9)
PHB33H56	0.85 (4)	0.71 (5)	0.52 (3)	0.58 (10)	1.07 (3)	1.64 (7)
Ngoyi	1.43 (9)	1.33 (9)	1.17 (1)	0.92 (2)	0.66 (2)	0.55 (4)
Sahara	1.37 (2)	1.19 (2)	0.17 (9)	0.84 (5)	0.68 (5)	0.86 (5)
PHB 3442	0.80 (5)	0.65 (3)	0.47 (5)	0.61 (4)	0.64 (10)	1.53 (10)
PAN 6Q408	0.42 (10)	0.48 (10)	0.48 (4)	0.50 (3)	0.60 (7)	0.63 (8)

Initial primary root length stress tolerance index: IPRLSTI; Final primary root length stress tolerance index: FPRLSTI; Net root growth stress tolerance index: NRGSTI; Shoot length stress tolerance index: SLSTI; Lateral seminal root length stress tolerance index: LSRLSTI; Lateral root number stress tolerance index: LRNSTI; *: rank into brackets.

3.6.1.6 Pearson correlation coefficients between shoot length and stress tolerance indices estimated for the haematoxylin assay

Strong, positive and highly significant correlation coefficients were found between Al SL and SLSTI (+0.64), as well as between Al SL and Al HS (+0.5) (Table 3.4). Very strong, positive and highly significant correlation coefficients were found between were also observed between FPRLSTI and IPRLSTI (+0.90); between LRNSTI and LSRLSTI (+0.7); SLSTI and IPRLSTI (+0.53); and SLSTI and FPRLSTI (+0.43) (Table 3.4). Positive and significant correlation coefficients were observed between Al HS and, IPRLSTI (+0.5), as well as between Al HS and NRGSTI (+0.42), whereas a negative and significant correlation coefficient was observed between Al HS and LRNSTI (-0.4). Since shoot length under Al toxicity (Al SL) can be considered as a proxy for total above ground biomass that is usually correlated with yield, shoot length stress tolerance index (SLSTI) and the haematoxylin staining score (Al HS) can be used for indirect selection for Al toxicity in the laboratory.

Table 3.4 Pearson correlation coefficients between shoot length and stress tolerance indices estimated for the Haematoxylin assay

	Al SL	IPRLSTI	FPRLSTI	NRGSTI	SLSTI	LSRLSTI	LRNSTI	Al HS
Al SL	1	0.16NS	0.06NS	0.09NS	0.64***	-0.17NS	-0.15NS	0.5**
IPRLSTI		1	0.90***	0.09NS	0.53**	0.22NS	-0.05NS	0.5**
FPRLSTI			1	0.23NS	0.43*	0.27NS	-0.08NS	0.4NS
NRGSTI				1	0.16NS	0.10NS	-0.07NS	-0.2NS
SLSTI					1	0.2NS	0.09NS	0.42*
LSRLSTI						1	0.7***	0.03NS
LRNSTI							1	-0.4*
Al HS								1

Al shoot length: Al SL; initial primary root length stress tolerance index: IPRLSTI; final primary root length stress tolerance index: FPRLSTI; net root growth stress tolerance index: NRGSTI; shoot length stress tolerance index: SLSTI; lateral seminal root length stress tolerance index: LSRLSTI; lateral root number stress tolerance index: LRNSTI; Al HS: haematoxylin staining score under Al condition, ***: significant at >0.1%; **: significant at 1%; *: significant at 5%; NS: non-significant.

3.6.2 Pot culture assay

3.6.2.1 Mean square of variables and indices estimated under limed and unlimed conditions in the glasshouse

Under unlimed conditions, significant differences were observed per site for seedling vigour index (USVI), Dickson quality index (UDQI), dry matter (UDM); plant height (UPH), leaf area under index (ULA) and stem diameter (USD) (Table 3.5). Under limed conditions, significant differences were observed per site for plant height (LPH), leaf area (LLA), and stem diameter (LSD) (Table 3.6.2.1). Under unlimed conditions, varieties expressed significant differences for USVI, UPH, and ULA. Under limed conditions, varieties expressed significant differences for LPH, LLA and LSD. The site by variety interaction was significant for USVI, UPH, and ULA under unlimed conditions. On the other hand, site by variety interaction was significant for LPH, and LLA under limed conditions.

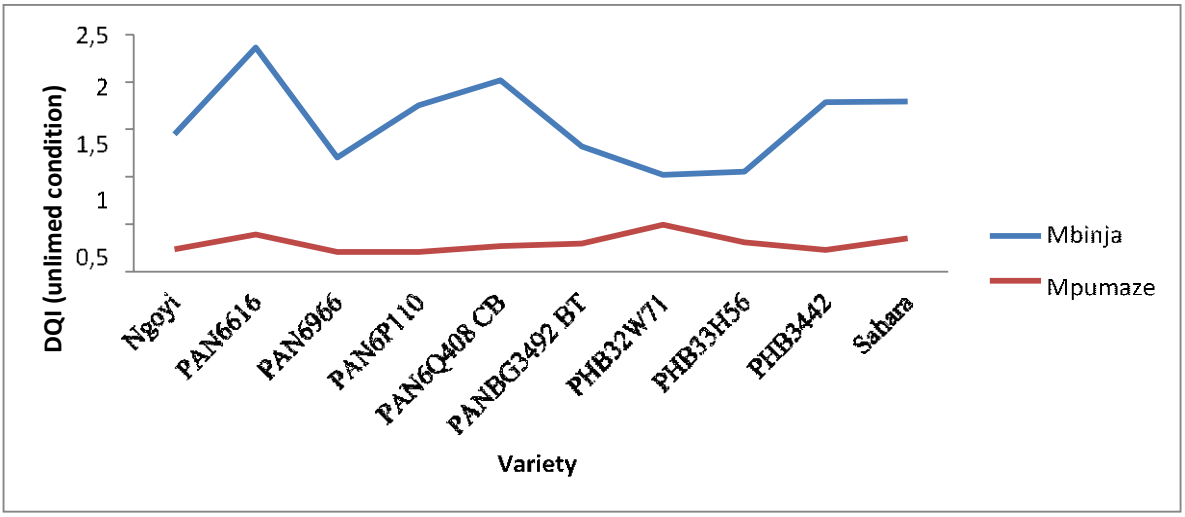
Table 3.5 Mean square of variables and indices estimated using the pot culture assay

Source	D F	USVI	LSVI	UDQI	LDQI	SLS TI	RLS TI	LAS TI	UDM	LDM	UPH	LPH	ULA	LLA	USD	LSD
Site	1	10410***	1611N S	24.48***	177.2NS	0.02NS	0.34NS	0.05NS	15.44**	570.7NS	6507***	6170***	3266***	8916***	34.2***	35***
Variety	9	309*	13700NS	0.29NS	77.3NS	0.13NS	0.35NS	0.49NS	3.17NS	531.2NS	48***	45.4***	67279**	153***	0.5NS	1.54*
Replication	2	148NS	10830NS	0.16N S	76.3NS	0.15NS	0.35NS	0.58NS	2.22NS	492.2NS	9.4NS	13.8NS	5611NS	10.4NS	1.95**	7.12**
Site*variety	9	358**	16017 NS	0.31N S	79.5N S	0.15NS	0.03NS	0.16NS	3.65NS	590.2NS	46***	503***	67527***	158***	0.48 NS	0.83 NS
Error	38	117	14235	0.24	79.6	0.12	0.28	0.35	1.89	555.9	11.24	5.6	4458	32.6	0.26	0.68

USVI: seedling vigour index under unlimed conditions; LSVI: seedling vigour index under limed conditions; UDQI: Dickson quality index under unlimed conditions; LDQI: Dickson quality index under limed conditions; SLSTI: shoot length stress tolerance index; RGSTI: root growth stress tolerance index; LASTI: leaf area stress tolerance index; UDM: dry matter under unlimed conditions; LDU: dry matter under limed conditions; UPH: plant height under unlimed conditions; LPH: plant height under limed conditions; ULA: leaf area under index unlimed conditions; LLA: leaf area under limed conditions; USD: stem diameter under unlimed conditions; LSD: stem diameter under limed conditions. DF: Degrees of freedom.

3.6.2.2 Variation of Duncan quality index (DQI) and seedling vigour index (SVI) of genotypes per village

Under soil acidity stress, DQI and SVI were able to discriminate the varieties evaluated per soil collected from the two different villages (Figure 3.3). The most tolerant genotype in soils collected from Mbinja was PAN6616 with the highest DQI, while genotype PHB32W71 was most tolerant in soils from Mpumaze. However, there were no significant differences between varieties when DQI was considered under limed and unlimed conditions. In soils from Mpumaze, PHB 32W71 was the most tolerant with the highest SVI, whilst genotype PAN6616 was most tolerant in soils from Mbinja when this index was used.



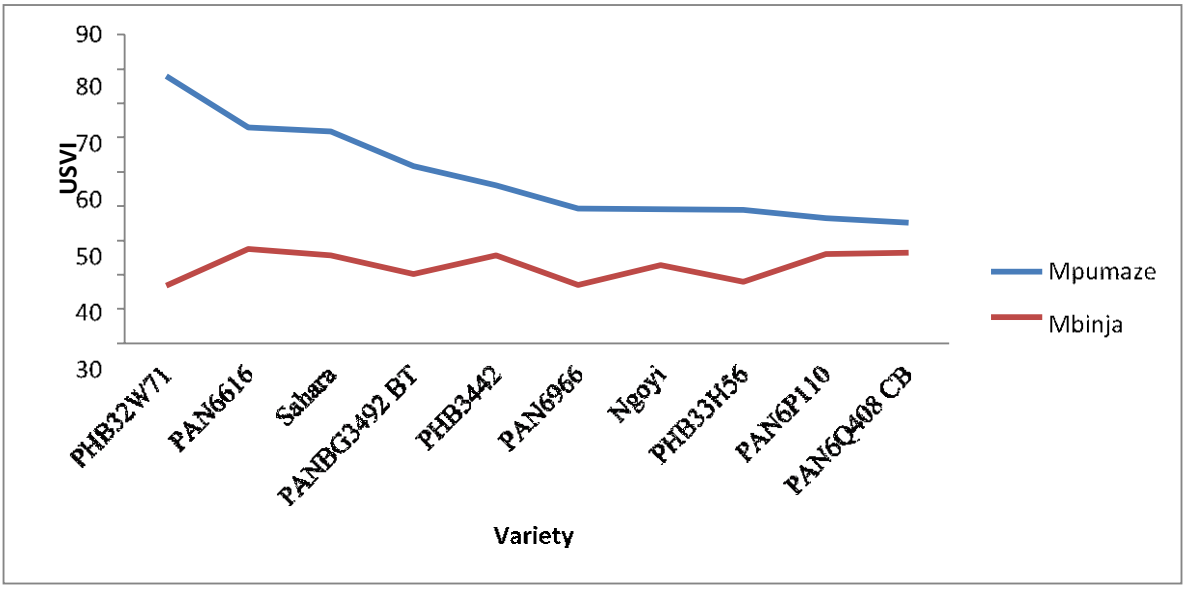


Figure 3.3 Variation of Duncan quality index (a) and seedling vigour index (b) of genotypes per village under acidity stress

3.6.2.3 Ranking of genotypes based on seedling vigour index (SVI)

Varieties were ranked using the SVI under unlimed conditions since significant differences were observed between them (Table 3.6). The top five most tolerant genotypes ranked according to their high seedling vigour index ranking under low soil pH in soils sampled at Mpumaze were PHB32W71, PAN6616, Sahara, PANBG3492 BT and PHB3442. On the other hand, the top five most tolerant genotypes in soils from Mbinja village were PAN6616, PAN6Q408 CB, PAN6P110, PHB3442 and Sahara (Table 3.6). The common varieties found among the top five in soils from the two villages were PAN6616, Sahara and PHB3442. These varieties could be considered the most tolerant genotypes under acidic soils collected from the two villages.

Table 3.6 Ranking of genotypes based on the seedling vigour index (SVI) under the soil acidity stress environment

Site1	Variety	USVI	Rank	Site2	USVI	Rank
Mpumaze	PHB32W71	77.83	1	**Mbinja**	16.9	10
Mpumaze	PAN6616	62.95	2	**Mbinja**	27.43	1
Mpumaze	Sahara	61.71	3	**Mbinja**	25.54	5
Mpumaze	PANBG3492 BT	51.71	4	**Mbinja**	20.11	7
Mpumaze	PHB3442	46	5	**Mbinja**	25.58	4
Mpumaze	PAN6966	39.32	6	**Mbinja**	17.03	9
Mpumaze	Ngoyi	39.11	7	**Mbinja**	22.9	6
Mpumaze	PHB33H56	38.95	8	**Mbinja**	17.95	8
Mpumaze	PAN6P110	36.42	9	**Mbinja**	26.07	3
Mpumaze	PAN6Q408 CB	35.24	10	**Mbinja**	26.3	2

USVI: seedling vigour index under soil acidity stress/ unlimed soil

3.6.2.4 Correlation between variables and indices

The seedling vigour index under unlimed conditions (USVI) showed highly positive and significant correlation coefficients with plant height (UPH) (+0.8), leaf area (ULA) (+0.8), and stem diameter (USD) (+0.7) (Table 3.7). Under unlimed conditions, dry matter (UDM) was highly and positively correlated with the Dickson quality index (UDQI) (+0.63), leaf area stress area tolerance index (LASTI) (+0.43), and seed vigour index (USVI) (O.39). LDQI was highly and positively correlated with LDM (0.99). Plant height under unlimed conditions (UPH) was highly and positively correlated with ULA (+0.95), and USD (+0.82). Significant and positive correlation coefficients were observed between LPH and LLA

(+0.93), and LSD (+0.71). Therefore, plant height, leaf area and stem diameter could be used for indirect selection for tolerance to soil acidity under glasshouse conditions.

Table 3.7 Correlation coefficients between variables and indices estimated in the glasshouse

	USVI	LSVI	UDQI	LDQI	SLSTI	RLSTI	LASTI	UDM	LDM	UPH	LPH	ULA	LLA	USD	LSD
USVI	1	0.02NS	-0.36**	-0.19NS	0.15NS	0.29*	0.25NS	0.39**	-0.13NS	0.8***	0.75***	0.8***	0.7***	0.7***	0.48***
LSVI		1	-0.19NS	0.95***	-0.027NS	-0.09NS	-0.16NS	-0.1NS	0.98***	0.07NS	0.09NS	0.10NS	0.11NS	-0.06NS	0.06NS
UDQI			1	-0.01NS	-0.21NS	-0.08NS	0.26NS	0.63***	-0.05NS	-0.72***	-0.7***	-0.64***	-0.63***	-0.50***	-0.42***
LDQI				1	0.009NS	-0.11NS	-0.12NS	-0.1NS	0.99***	-0.17NS	-0.18NS	-0.15NS	-0.16NS	-0.25NS	-0.13NS
SLSTI					1	0.08NS	0.2NS	0.15NS	-0.008NS	0.23NS	-0.11NS	0.04NS	-0.08NS	0.08NS	-0.13NS
RLSTI						1	0.16NS	0.16NS	-0.11NS	0.15NS	0.13NS	0.11NS	0.10NS	0.19NS	0.15NS
LASTI							1	0.44***	-0.13NS	0.01NS	-0.14NS	-0.08NS	-0.27*	0.04NS	-0.31*
UDM								1	-0.1NS8	-0.09NS	-0.16NS	-0.008NS	-0.12NS	-0.01NS	-0.08NS
LDM									1	-0.10NS	-0.1NS	-0.08NS	-0.08NS	-0.2NS	-0.09NS
UPH										1	0.9***	0.95***	0.85***	0.82***	0.61***
LPH											1	0.95***	0.93***	0.78***	0.71***
ULA												1	0.91***	0.81***	0.69***
LLA													1	0.71***	0.73***
USD														1	0.64***
LSD															1

USVI: seedling vigour index under unlimed condition; LSVI: seedling vigour index under limed condition; UDQI: Dickson quality index under unlimed condition; LDQI: Dickson quality index under limed condition; SLSTI: shoot length stress tolerance index; RLSTI: root length stress tolerance index; LASTI: leaf area stress tolerance index; UDM: dry matter under unlimed condition; LDU: dry matter under limed condition; UPH: plant height under limed condition; LPH: plant height under limed condition; ULA: leaf area under unlimed condition; LLA: leaf area under limed condition; USD: stem diameter under unlimed condition; LSD: stem diameter under limed condition.

3.6.3 Comparison of outcomes from laboratory and glasshouse studies

Some similar variables and indices estimated in the laboratory and glasshouse experiments were used to generate the mean square values in the two different study conditions. It was shown that there were significant differences for all the indices in the two environments (Table 3.8). However, the root growth stress tolerance index (RGSTI) additionally showed significant differences among varieties and its environment by variety interaction was also significant. On the basis of these observations, the RGSTI was considered to be the best index to discriminate differences among genotypes under laboratory and glasshouse conditions.

Table 3.8 Mean squares of indices and variables estimated in the laboratory and under glasshouse conditions

Source of variation	DF	SLSTI	RGSTI	Al SL	No Al SL
Replication	2	0.12^{ns}	0.06^{ns}	2.87^{ns}	0.63^{ns}
Environment	1	1.84***	3.51***	19.63***	6.60*
Variety	9	0.10^{ns}	0.34***	2.41^{ns}	1.49^{ns}
Environment*Variety	9	0.03^{ns}	0.29**	1.65^{ns}	1.83^{ns}
Error	38	0.11	0.07	1.22	1.12

SLSTI: Shoot length stress tolerance index, RGSTI: root growth stress tolerance index
Al SL: Shoot length under aluminium toxicity, No Al SL: shoot length under control condition, DF: degree of freedom

3.6.4 Ranking of genotypes for each assay using the root growth stress tolerance index (RGSTI)

The ranking of genotypes based on the RGSTI showed that Ngoyi, PANBG3492 BT, PAN 6Q408 and PHB 3442 were among the top five most tolerant genotypes under the laboratory and the glasshouse conditions (Table 3.9). Sahara was the second most tolerant genotype in the glasshouse but was not among the top five most tolerant varieties in the laboratory. Similarly, PHB33H56 was the third best genotype in the laboratory though it was not in the top five under glasshouse conditions.

Table 3.9 Ranking of genotypes across environments based on the root growth stress tolerance index (RGSTI)

Variety	Glasshouse	Rank	Laboratory	Rank
Ngoyi	0,89	5	1,17	1
BG3492 BT	0,94	4	1,005	2
PHB33H56	0,83	7	0,52	3
PAN 6Q408	0,99	3	0,48	4
PHB 3442	1,73	1	0,47	5
PAN 6P110	0,82	8	0,39	6
PHB32W71	0,74	10	0,28	7
PAN 6966	0,86	6	0,24	8
Sahara	1,02	2	0,17	9
PAN 6616	0,77	9	0,04	10

3.7 DISCUSSION

Significant differences were observed per site for the seedling vigour index (USVI) under unlimed conditions while non-significant differences were observed using the Dickson quality index (UDQI). Under soil acidity stress, the USVI was therefore able to discriminate the varieties evaluated per type of soil collected in the different villages. At Mpumaze, PHB32W71 was the most tolerant with the highest USVI while at Mbinja, PAN6616 had the highest USVI. The results of this study confirm the findings of Foy *et al.* (1988) and Musunda *et al*. (2012) who reported that plant species vary widely in their ability to grow on acidic soils and different agro-ecologies. Adebisi *et al*. (2014) stated that seedling vigour index (SVI) is an imperative quality parameter which needs to be evaluated to supplement germination and viability tests to gain insight into the performance of a seed lot in the field. In this current study, varieties expressed significant differences for USVI, ULA, LSD and UPH. Several authors reported that seed lots that produce taller seedlings are considered more vigorous than the seed lots that produced shorter seedlings and poor SVI may be due to their relatively small root length, leading to insufficient water and nutrient uptake under aluminium toxicity conditions (Prasanna, 2013; Adebisi *et al*., 2014; Lin, *et al*., 2018). On the other hand, tall and slender seedlings have lower survival rates after transplanting (Jacobs *et al*., 2005). The seedling vigour index under unlimed conditions (USVI) showed a highly positive and significant correlation with plant height (UPH) (+0.8). Adebisi *et al.* (2014) reported a positive and significant correlation coefficient between seedling vigour and plant height (0.68) under

stress conditions. It has been reported that plants with high SV are healthy, exhibit strong growth and vitality, have dominant stems, occupy large root zones, have balanced shoot/root ratio and are able tolerate stressful environments (Wightman, 1999).

Leaf area (LA) has been used as a land surface biophysical parameter for almost all models simulating ecosystems processes (Song, 2012). The Dickson quality index under unlimed conditions (UDQI) was highly and negatively correlated with ULA (-0.64) showing that LA is an inaccurate predictor of SV in this study. The results of this study contradict the findings of Lin *et al*. (2018) who reported a strong and significant correlation between DQI and LA, showing that LA is an accurate and non-destructive predictor of SV. However, these indices (UDQI and ULA) were always subject to more variation from one experiment to another which may have been caused by the different physiological states of the plants (Cancado *et al*., 1999). Therefore, the results are likely to differ if the experiment is repeated using different plant materials. Measurement of plant dry mass is often used as an indicator for seedling survival (Lin *et al*., 2018). Dry mass is representative of the net gain of photosynthesis, and plants with higher dry mass have better growth potential and are of better quality (Manas *et al*., 2009). The Dickson quality index under unlimed conditions (UDQI) was highly and positively correlated with dry matter (UDM) (+0.68) in this study. Similar findings were reported by Lin *et al*. (2018) who obtained a positive correlation (0.86) between DQI and DM.

In terms of ranking, PHB32W71, PAN6616, Sahara, PANBG3492 BT and PHB3442 were the top five most tolerant genotypes ranked according to their high seedling vigour index under low soil pH in soils from Mbinja. Use of soils from Mpumaze revealed that the top five most tolerant genotypes according to their seedling vigour index were PAN6616, PAN6Q408 CB, PAN6P110, PHB3442 and Sahara. Varieties PAN6616, Sahara and PHB3442 were the most tolerant genotypes under low soil pH across soils from the two villages. Similar results were reported by Van Averberke (1991) who found that a single maize variety responded differently in different soil ecotypes in the same location (soil factors) and in different locations with the same soil ecotypes (environmental factors). Musunda *et al*. (2012) have indicated that the varieties that are found to be tolerant in one type of soil may not necessarily be tolerant in another. The environmental factors that affect the phenotypic response of maize in a given location include rainfall, temperature, biotic factors and soil conditions, etc, and these can have either a collectively positive or negative effect on the phenotypic response of a maize plant (Admassun *et al*., 2008). The

best phenotypic index used to characterize a genotype's tolerance or sensitivity under glasshouse conditions was earlier found to be differences in root length (Adebisi *et al.*, 2014).

In this current study, the ranking of genotypes based on the root growth stress tolerance index showed that Ngoyi, PANBG3492 BT, PAN 6Q408 and PHB 3442 were among the top four most tolerant genotypes under laboratory and glasshouse conditions. These results are in agreement with Mazzocato *et al*. (2002), who also managed to identify distinct groups (Al tolerant and sensitive) when maize genotypes were assessed under Al stress conditions. Glasshouse and laboratory assays consistently discriminated similar tolerant maize genotypes of based on the RGSTI. Similar results were obtained by Paterniani and Furlani (2002). Therefore, the root growth stress tolerance index was the best index that was able to discriminate differences among genotypes in the laboratory and under glasshouse conditions.

Positive and significant relationships were observed between shoot length and shoot length stress tolerance index, as well as between shoot length and the haematoxylin score under laboratory conditions. Shoot length stress tolerance index and the haematoxylin score can therefore be used for indirect selection for tolerance to Al toxicity when one is using the haematoxylin assay. On the other hand, plant height, leaf area and stem diameter were found to be useful for indirect selection for tolerance to soil acidity under glasshouse conditions.

3.8 CONCLUSIONS AND RECOMMENDATIONS

Glasshouse and laboratory assays identified similar tolerant genotypes of maize based on RGSTI. The tolerant genotypes were namely Ngoyi, PAN BG3492 BT, PAN 6Q408 and PHB 3442. Therefore, the root growth stress tolerance index was the best index that could identify tolerant genotypes in the laboratory and in the glasshouse under stress conditions. The tolerant genotypes are recommended for further evaluation under field conditions to ascertain their yield potential under soil acidity. Shoot length stress tolerance index and the haematoxylin score were found to be useful for indirect selection for tolerance to Al toxicity in the laboratory. On the other hand, plant height, leaf area and stem diameter were found to be useful for indirect selection for tolerance to soil acidity under glasshouse conditions.

CHAPTER 4

FIELD SCREENING OF MAIZE GENOTYPES FOR TOLERANCE TO ACIDIC SOILS AT MHLONTLO MUNICIPALITY, EASTERN CAPE

ABSTRACT

Tolerance of 10 maize (*Zea mays* L.) genotypes to soil acidity was investigated by growing them in acidic soils in the field. Two sites were used, namely Mbinja and Mpumaze. With the use of limed and unlimed field plots, the trial was set up in a randomized complete block design with three replications. Kernel yield and other parameters were recorded. Selection of tolerant genotypes was also done in using three indices namely the harmonic mean (HM), stress tolerance index (STI) and stress susceptibility index (SSI). Significant differences were observed among genotypes for ear height (EH), ear diameter (ED), stem diameter (SD), and leaf area (LA), number of kernels per row (NKR), number of rows per cob (NRC), plant height (PH), 1000 kernel weight (KWT) and grain yield (GY) (P ≤0.1%) under unlimed conditions. under field conditions. The best selection indices were HM and STI since they were able to identify the stress tolerant genotypes. The top three tolerant genotypes at Mbinja were PHB33H56, PANBG3492 BT and PHB32W71, while at Mpumaze the top three were PAN6P110, PAN6Q408 and PHB3442. The highly significant and positive correlations observed between ear diameter (0.9), ear length (0.9), and leaf area (0.7), with1000 kernel weight indicated that such traits could be useful in identifying genotypes that are tolerant to soil acidity under field conditions. Varieties that were observed to be tolerant in the different sites are recommended for further evaluation in several sites to confirm their tolerance.

Key words: Maize, soil acidity, stress indices.

4.1 INTRODUCTION

Maize (*Zea mays* L.) is South Africa's staple food crop, and it is extensively grown in the Eastern Cape (EC) (PROVIDE, 2009). Coincidentally, the province has one of the highest poverty and food insecurity incidences in South Africa, with more than 65% of the inhabitants living in rural areas (PROVIDE, 2009). According to Pauw (2006), a considerable number of rural dwellers survive on semi-subsistence agriculture, and are considered to be resource-poor. Maize is the main summer crop grown by most, if not all, farmers (Sibanda, 2010). However, resource-poor farmers are currently unable to produce sufficient grain to meet their household consumption requirements in the EC province (Bennett, 2002; ISER, 2001).

There are numerous factors that contribute to food insecurity in the smallholder sector. Yield decrease in most dryland maize growing areas occurs because seasonal rainfall distribution is unreliable (Du Toit *et al*., 2002). Smallholder farmers are more susceptible to climate variability and its impact on maize yield than are commercial farmers (CMMYT, 1999). The EC also has one of the highest provincial indices of soil degradation, and this is evident by its low soil fertility status (Mandiringana *et. al.*, 2005). Silwana (2000) reported that maize is usually grown under poor soil-fertility conditions in the EC, such as low nitrogen (N), phosphorus (P) and soil acidity. Low soil pH, which affects the availability of micro- and macro-nutrients, is a very important yield-limiting factor in the high rainfall areas of the EC province (Mandiringana *et. al.*, 2005; Gichangi, 2007). Acidic soils with high acid saturation make phosphorus, nitrogen, potassium, sulphur, magnesium and calcium less available to plants (Larson, 2008). Acidic soils do not only reduce maize growth, but are also accompanied by sparse foliage and symptoms of nutrient deficiency that tend to be more important factors affecting maize yields (ARC, 1995; Narro *et al.*, 2001).

Farmers have been consistently producing low maize yields due to soil acidity in affected areas (Pineros *et al*., 2005; Tolra *et al*., 2005; Gudu *et al*., 2001). Maize genotypes that are tolerant to acidic soil would be beneficial to smallholder farmers who do not have the resources to buy lime to ameliorate the problem. The use of maize varieties that are tolerant to acidic soil constitutes a coherent and lasting alternative for the production of greater yields under low soil pH and the prevention of huge losses of grain yields often observed with maize varieties that are sensitive to acidic soils (Horst *et al*., 1997). In the

end, the use of tolerant varieties is cheaper, maintainable and more environmentally friendly.

Different stress tolerance indices showing the effects of stress have been recommended based on grain yield under stress conditions as compared to non-stress conditions (Francisco *et al*., 2010). Rosielle and Hamblim (1981) defined mean productivity (MP) as the average yield of a genotype under constructive stress and optimal conditions. Fischer and Maurer (1978) proposed the use of stress susceptibility index (SSI) for evaluating yield stability and to determine the change in both potential and actual yield in variable environments. Fernandez (1992) defined the stress tolerance index (STI), which can be used to identify genotypes that produce a high yield under both stress and non-stress conditions. Fernandez, (1992) also proposed another yield based estimation of stress tolerance, which is called the harmonic mean (HM).

4.2 OBJECTIVES

The main objective of the current study is to:

- Determine the response of maize varieties to acidic soils under field conditions.

The specific objectives are:

- To identify maize genotypes that are tolerant to acidic soils under field conditions.
- To identify secondary traits that are associated with tolerance of maize genotypes to acidic soils under field conditions.

4.3 HYPOTHESES TO BE TESTED

- All varieties tested in this experiment are tolerant to acidic soils.
- There are secondary traits associated with tolerance to acidic soils among tested maize genotypes.

4.4 MATERIALS AND METHODS

4.4.1 Site description

The study was conducted from October 2013 to July 2015 for two seasons in the Mhlontlo Local Municipality of the Eastern Cape (O.R. Tambo District), in the rural areas of the Tsolo and Qumbu sub-district. The characteristics of the two sites that were used are shown in Table 4.1.

Table 4.1 Agro-climatic characteristics of the experimental sites

Collection site	Soil type	GPS location	Clay %	pH (KCl)	Acid saturation %	Maximum annual temp. for the whole year	Minimum annual temp. for the whole year	Average annual temp for 15 years	Annual average rainfall
Mbinja	Sepane form	31° 43´´S 28° 43´´E	29%	3.85	36	24°	8°	16°	627 mm
Mpumaze	Oudtshoorn form	31° 08´14" S 28° 53´40" E	7%	4.15	24	24.2°	10.5°	17.4°	637 mm

GPS: Geographical positioning system, %: percent, mm: millimetres

4.5 TRIAL ESTABLISHMENT

4.5.1 Plant materials

A total of 10 maize varieties collected from Pannar and Pioneer, as well as a local landrace collected from the farmers of Mbinja and Mpumaze were used in this study. The ten maize varieties are shown in Table 3.1, in Chapter 3.

4.5.2 Experimental procedures and design

The experimental plots consisted of five rows per variety, each row measuring 5 m in length. The 10 varieties were planted in a randomised complete block design (RCBD) with three replicates under two different environments. The environments consisted of an acid soil and a limed environment, the latter being used as a control. Plants were spaced 100 cm and 30 cm, for inter row and intra row spacings respectively. This gave a plant population of 33 3333 plants/ha. An area of 148 m^2 was therefore used for this experiment.

4.5.3 Trial management

Land preparation was done using conventional ploughing. Weeds were controlled using pre-planting and post-emergence herbicides. Before planting, a pre-emergence herbicide, namely Alachlor 480CS, was applied at the recommended rate of 5 l/ ha (a.i. chloroacetanilide 480 g/l). From two weeks after crop emergence, basagran (a.i. bendioxide 480 g//) and atrazine 500SC (a.i. atrazine 500 g//) were applied at the

recommended rates of 2 *l*/ha to control broad leafed weeds and nutsedge when necessary. Cut worms (*Agrotis segetum*) and the maize stalk borer (*Buseola fusca*) were controled using dursban at 3 *ml*/5*l*. All agro-chemicals were applied using a knapsack sprayer. The idea was to test the varieties under neutral soil pH conditions, and then compare their performance with those in acidic soil. Basal fertilizer with an N: P: K ratio of 2:3:4 (30) was applied at a rate of 185 kg/ha at planting to give a nutrient ratio (kg) of 12.4 N: 18.5 P: 24.6 K. Lime ammonium nitrate (LAN) (28% N) was applied at a rate of 185 kg/ha 6 weeks after crop emergence (WACE). Therefore, N fertilizer was applied at a rate of 64 kg/ha.

4.6 DATA COLLECTED

Agro-morphological characteristics that are listed in the Table 4.2 were recorded following standard procedures (CIMMYT, 1995).

Table 4.2 Agro-morphological characteristics that were collected at Mbinja and Mpumaze

Trait	Description	Unit of measurement
Plant height	From ground level to the base of the tassel using flexible tape. Height was collected after the milk stage	cm
Stem diameter	Measured just below the ear using a Vanier calliper	mm
Leaf area	Was estimated as a product of the leaf length (L) and the widest middle portion of the leaf. Width of the leaf will be corrected to 0.75, as described by Sexena and Singh (1965) as follows: LA=0.75 (LxW)	cm^2
Grain yield	Yield adjusted at 12.5% moisture content. Grain yield= (100-moisture content/87.5 x yield/ha.	Kg/ha
1000 Kernel weight	Weight of 1000 kernels	g
Kernel per row	Count number of kernels per row on maize ear.	Visual counting
Ear length	From the tip to the top of the cob using flexible tape.	cm
Ear diameter	Measuring the circumference of the ear using a flexible tape.	mm
Ear height	From ground level to node bearing the uppermost ear using a flexible tape. After milk stage.	cm
Tassel length	Distance between tassel branches.	cm
Peduncle length	Distance between leaf sheath and tertiary ramification. After milk stage.	cm

Stress tolerance indices were calculated as follows:

- The **Stress susceptible index** (SSI) was calculated according to the formula by Fischer and Maurer (1978): **SSI=1-(Ys / Yp) /SI, while S I=1- (Ýp / Ýp)**
- The **Stress tolerance index** (STI) was calculated according to the formula by Fernandez (1992): **STI=1-(Ys X Yp)/(Ýp)2**
- The **Harmonic mean** (HM) was calculated according to the formula by Fernandez (1992): **HM= 2(Yp X Ys)/ (Yp + Ys)**

Where: Yp= Normal condition; Ys= Stress condition

4.7 DATA ANALYSIS

SAS version 9.2 was used for data analysis. An initial analysis of variance (ANOVA) was performed on all the collected parameters for each genotype (G) and the Tukey's test was used to separate means that were significantly different from each other. Subsequently, combined ANOVA was conducted over sites and seasons. A correlation analysis was also conducted between traits that were recorded during the field trials.

4.8 RESULTS

4.8.1 Variance estimates for recorded parameters and tolerance indices

The analysis of variance showed significant differences among genotypes evaluated for most of the parameters recorded except for lengths of the ear, peduncle and tassel length (Table 4.3). Additionally, parameters collected were statistically significant over the years of evaluation except for the number of kernels per row. Significant differences were also observed for traits collected across villages except for ear height, plant height and grain yield. The significance of mean squares for year by variety interaction, village by variety interaction and year by village by variety interaction implied that for some traits collected, the varieties responded differently per village over the years of evaluation (Table 4.3). The stress tolerance index (STI) and harmonic mean index (HARMI) expressed significant differences for varieties evaluated over years in the villages whereas the stress susceptibility index (SSI) was not significant for all the factors considered (Table 4.4). The non-significance of the SSI implied that all the genotypes expressed similar levels of tolerance to low soil pH based on this index.

Table 4.3 Analysis of variance for all the parameters collected of the ten genotypes tested across two locations over years under low soil pH

Source of variation	DF	Ear diameter	Ear length	Kernel per row	Rows per cob	Stem diameter	Ear height	Leaf area	Peduncle	Tassel	Plan height	KWT
Year	1	49655***	665448***	103*	1.1NS	13387***	37835***	2530641***	5174***	858***	470845***	118678***
Vill	1	41982***	21307***	3252***	9*	159*	252NS	174009***	2826***	1166***	1.2NS	957***
Rep	2	298**	390*	35NS	0.7NS	661***	89NS	111369***	22NS	25NS	459NS	47NS
Var	9	185***	130NS	114***	40***	145***	1789***	28504***	7NS	36NS	1887***	63*
Enviro	1	99NS	80NS	0.009NS	4.2NS	30NS	167NS	84NS	34NS	0.5NS	42NS	0.1NS
Year*Var	9	23NS	112NS	42*	2.8*	37NS	598***	7271NS	15NS	58*	3314***	171***
Vill*Var	9	27NS	356**	23NS	1.1NS	20NS	30NS	6762NS	4NS	22NS	216NS	64*
Var*Enviro	9	99*	171NS	19NS	1.3NS	29NS	45NS	11909NS	4NS	16NS	148NS	21NS
Year*Vill*Var	10	4984***	2038***	31NS	1.5NS	136***	277*	11907NS	299***	20NS	726NS	189***
Error	158	50	123	19	1,4	32	130	6910	12	26	422	28

***: Significant at >0.1%; **: Significant at 1%; *: Significant at 5%; ns: non-significant; KWT- Kernel weight; DF-

Table 4.4 Variances of stress tolerance indices that were estimated for the two villages

Source of variation	DF	STI	HARMI	SSI
Replication	2	0,12*	26,90NS	43483NS
Variety	9	0,16***	32,39*	35470NS
Year	1	1045***	59746***	3973NS
Village	1	2,06***	512***	11844NS
Var*Year	9	0,24***	93***	20455NS
Var*Vill	9	0,06NS	33,8NS	26123NS
Var*Year*Vill	10	0,29***	88,2***	18130NS
Error	78	0,33	14.52	40755

***: Significant at >0.1%; **: Significant at 1%; *: Significant at 5%; ns: non-significant; DF: Degree of freedom, STI: Stress tolerance index, HARMI: Harmonic mean index, SSI: Stress susceptibility index

4.8.2 Mean performance of genotypes evaluated

The varieties evaluated showed mean grain yields that varied from 534.13 kg/ha (Ngoyi) to 821.81 kg/ha (PAN6Q408). However, part of the yield data was lost, such that it could not be used for further assessment of varietal performances. Mean plant height ranged from 136.41 cm (Ngoyi) to 163.72 cm (PANBG3492 BT); mean leaf area varied from 275.23 cm^2 (Ngoyi) to 395.45 cm^2 (PAN6P110); ear height varied from 59.36 cm (Ngoyi) to 89.26 cm (Sahara); stem diameter ranged from 26.67 mm (Ngoyi) to 34.38 mm (PAN6616); mean kernel weight of 1000 grains varied from 46.67 g (PAN6616) to 50.43g (PHB33H56); the number of kernel rows per cob ranged from 10 (Ngoyi) to 14 (PAN6Q408 and PANBG3492 BT); the number of kernels per row varied from 24 (Ngoyi) to 31 (PHB3442); and ear diameter ranged from 47.09 mm (Ngoyi) to 56.09 mm (PAN6P110) (Table 4.5).

Table 4.5 Mean performance of traits collected across villages over the years

Variety	Ear diameter (mm)	Ear Length (cm)	Kernel per row	Row per ear	Kernel Weight (g)	Stem Diameter (mm)	Ear height (cm)	Leaf area (cm^2)	Peduncle length	Tassel length	Plant Height (cm)
Ngoyi	47,09	62,59	24	10	47	26,56	59,36	275,23	13,79	27,30	136,41
PAN6616	56,06	64,22	27	13	46,67	34,38	83,02	383,47	13,99	28,22	156,34
PAN6966	56,07	63,46	28	13	48,11	33,73	86,66	376,61	14,77	30,58	151,62
PAN6P110	56,09	67,42	29	13	50,10	33,90	75,88	395,45	14,17	28,54	141,61
PAN6Q408	54,36	66,66	32	14	49,07	33,46	77,04	392,87	14,27	28,00	149,45
PANBG3492 BT	55,54	62,67	30	14	50,35	29,99	68,77	348,25	14,87	29,23	163,72
PHB32W71	55,27	65,13	30	13	50,28	30,54	77,88	353,31	13,81	26,55	158,86
PHB33H56	54,55	66,42	29	13	50,43	29,82	76,77	350,74	13,97	26,89	161,58
PHB3442	55,64	69,44	31	13	49,75	30,50	80,53	351,08	15,23	28,59	159,20
Sahara	52,40	67,71	29	11	46,50	32,03	89,26	355,35	15,15	29,39	157,41

Cm: Centimetre, G: gram

4.8.3 Ranking of genotypes based on stress tolerance indices

The top three genotypes at Mbinja were PHB33H56, PANBG3492 BT and PHB32W71 according to their ranking based on STI and HARMI. At Mpumaze, the top three genotypes were PAN6P110, PAN6Q408 and PHB3442 in descending order (Table 4.6). The variety PAN6P110, which was ranked fist at Mpumaze, occupied the fifth place at Mbinja when using STI.

Table 4.6 Ranking of genotypes based on stress tolerance indices per village

Variety	Village	STI	Rank	HARMI	Rank	Village	STI	Rank	HARMI	Rank
PHB33H56	Mbinja	1,57	1	53,87	1	Mpumaze	1,16	5	46,89	5
PANBG3492 BT	Mbinja	1,53	2	52,30	2	Mpumaze	1,14	6	47,01	6
PHB32W71	Mbinja	1,51	3	52,07	3	Mpumaze	1,19	4	48,19	4
Ngoyi	Mbinja	1,32	6	52,05	4	Mpumaze	0,84	10	41,09	10
PHB3442	Mbinja	1,38	4	50,08	5	Mpumaze	1,21	3	49,23	3
PAN6P110	Mbinja	1,36	5	50,04	6	Mpumaze	1,28	1	49,72	1
PAN6966	Mbinja	1,31	7	49,94	7	Mpumaze	1,07	7	45,61	7
PAN6Q408	Mbinja	1,30	8	48,82	8	Mpumaze	1,25	2	49,06	2
Sahara	Mbinja	1,19	10	48,65	9	Mpumaze	0,98	8	43,71	8
PAN6616	Mbinja	1,22	9	48,58	10	Mpumaze	0,97	9	44,54	9

***: Significant at >0.1%; **: Significant at 1%; *: Significant at 5%; ns: non-significant; STI: Stress tolerance index, HARMI: Harmonic mean index

4.8.4 Correlation between parameters that were recorded

Some parameters showed strong positive and highly significant correlation coefficients (Table 4.7). These traits were kernel weight, which was highly correlated with ear diameter (+0.7), leaf area (+0.7) and ear length (+0.9). However, kernel weight was highly significantly and negatively correlated with stem diameter (-0.7), ear height (-0.6), peduncle length (-0.6), tassel length (-0.3) and plant height (-0.8). Plant height was highly and positively correlated with ear height (+0.8), stem diameter (+0.7) and peduncle length (+0.6) (Table 4.7). Therefore, leaf area, ear diameter and ear length could be used as indirect selection criteria for plant tolerance to low soil pH under field conditions. This is because they were highly significantly and positively correlated to kernel weight under soil acidity stress conditions. The number of rows per cob showed a relatively low correlation coefficient with all the parameters that were recorded.

Table 4.7 Correlation coefficient between traits recorded across the two villages

	Ear diameter	Ear length	Kernels per row	Rows per cob	Kernel weight	Stem diameter	Ear height	Leaf area	Peduncle length	Tassel length	Plant height
Ear diameter	1										
Ear length	0.4***	1									
Kernel/row	-0.3***	0.1*	1								
Row/cob	0.2**	-0.02NS	0.3***	1							
Kernel weight	0.7***	0.9***	-0.1NS	0.01NS	1						
Stem diameter	-0.3***	-0.7***	0.2**	0.1*	-0.7***	1					
Ear height	-0.4***	-0.6***	0.1*	0.2**	-0.6***	0.6***	1				
Leaf area	0.5***	0.8***	0.2**	0.1NS	0.7***	-0.4***	-0.4***	1			
Peduncle	-0.3***	-0.6***	-0.1NS	0.09NS	-0.6***	0.3***	0.6***	-0.6***	1		
Tassel	0.04NS	-0.3***	-0.06NS	0.1*	-0.3***	0.3***4	0.5***	-0.2***	0.6***	1	
Plant height	-0.5***	-0.8***	0.2**	0.1*	-0.8***	0.7***	0.8***	-0.6***	0.6***	0.4***	1

***: Significant at >0.1%; **: Significant at 1%; *: Significant at 5%; ns: non-significant

4.9 DISCUSSION

Variability was observed among the genotypes evaluated for leaf area in this study. Genotype PAN6P110 recorded the highest mean leaf area (395.45 cm^2), while Ngoyi recorded the lowest (275.23 cm^2). This indicates that all the genotypes expressed different levels of tolerance to low soil pH for leaf area. The results of this study compare favourably with those obtained by Bawa *et al.* (2015) who reported a tolerant genotype obtained the highest mean leaf area relative to other genotypes. Modarres *et al.* (1998) reported that plants with bigger leaf area tend to have higher yields than those with smaller leaf area. There was also a highly significant variation for plant height among the genotypes, and genotype PANBG3492 BT recorded the highest mean plant height (163.72 cm), while genotype Ngoyi recorded the lowest (136.41 cm). This supports the observations of Pandey *et al.* (2000) and Bawa *et al.* (2015) who pointed out that increases in stress conditions result in progressively less plant height. This might also explain the very high and significantly negative relationship that was observed between 1000 kernel weight and plant height. Based on the negative relationships with 1000 kernel weight, shorter plants with smaller grains, lower ear heights, shorter peduncles, thinner stems and shorter tassel lengths have higher chances of survival under soil acidity in the field.

The significance of mean squares for village by variety interaction was observed in this study for ear length and 1000 kernel weight. Van Averbeke (1991) found that a single maize variety responded differently in different soil ecotypes in the same location (soil factors) and in different locations with same environmental factors. The interaction also implied that the genotypes evaluated showed different responses in the two villages for ear length and 1000 kernel weight. At Mpumaze the highest means for these traits were recorded as compared to Mbinja. The significance of year by variety by village interaction for ear diameter, ear length, stem diameter, ear height, peduncle length and 1000 kernel weight implied that that varieties performed differently in each year and in each village. These results are supported by Admassun *et al*. (2008) who reported that environmental factors that affect the phenotypic response of maize include location, amount of rainfall, length of growing season, temperature, amount of precipitation per season, soil conditions, etc, and these can have either a collectively positive or negative effect on the phenotypic response of a maize plant.

The best selection indices must be able to distinguish genotypes that have superior performance under stress environments (Darnishzadek *et al*., 2010). In this current study, the best selection indices were HM and STI since they were able to identify the stress tolerant genotypes. The top three genotypes at Mbinja were PHB33H56, PANBG3492 BT and PHB32W71 while at Mpumaze the top three were PAN6P110, PAN6Q408 and PHB3442. The results of this study agree with the report from Khayatnezhad *et al.* (2011) who stated that genotypes with specific adaptation to certain sites can be identified through evaluation at diverse sites. This also confirms reports by Foy *et al*. (1988) and Musunda *et al*. (2012) who stated that the genotypes that are found to be tolerant in one type of soil may not necessarily be tolerant in another. The two sites might also be having different metal toxicities out of the three (Al, Fe, and Mn) that are known to occur in nature (Tandzi *et al*., 2018).

Additionally, in this study STI and HM were able to identify genotypes that have high tolerance and high yield under acidic stress conditions. The top two stress tolerant genotypes across the villages over years were PAN6P110 and PAN6Q408. A similar observation was reported by Fernandez (1992) and Zare (2010) who stated that mung bean genotypes which were selected based on STI showed high tolerance and high yield under stress conditions. Therefore, in this study STI and HM were shown to be the better indices in predicting superior performance under acidic stress conditions.

Kernel weight was highly significantly and positively correlated with ear diameter (+0.7), leaf area (+0.7) and ear length (+0.9). These results suggest the reliability of these traits (leaf area, ear diameter and ear length) as predictors of performance under low soil pH conditions. These traits can therefore be used as indirect selection criteria for maize tolerance to low soil pH under field conditions. The results agree with the findings of Majid *et al*., (2011) who reported that increasing ear diameter resulted in an increase in the number of rows per ear and consequent increases in the number of kernels per ear. In other words, the increase of ear diameter induced an increase in the kernel yield of maize. Monneveux *et al*., (2008) reported that genotypes possessing bigger kernels could help to increase grain yield. PAN6Q408 and PAN6P110 produced smaller tassels than PAN6696 but both genotypes produced more yield than PAN6696. Banziger *et al.* (2000) and Monneveux *et al.* (2008) suggested that varieties with smaller tassels could help to increase grain yield in stress prone environments. This is because less nutrients will be

partitioned to the smaller tassels which will benefit ear development. In this study, tassel length was also found to be significantly and negatively correlated to kernel weight.

4.10 CONCLUSIONS AND RECOMMENDATIONS

The top three genotypes at Mbinja were PHB33H56, PANBG3492 BT and PHB32W71 while at Mpumaze the top three were PAN6P110, PAN6Q408 and PHB3442. These varieties can be recommended for production in each of these sites. The varieties can also be recommended for evaluation in more soil acidity hot spots to determine their suitability of production in those sites. From this study, the high and positive correlations observed between ear length, leaf area, ear diameter and 1000 kernel weight indicated that such traits could be useful in identifying genotypes that are tolerant to soil acidity under field conditions. Additionally, the best selection indices were HM and STI since they were able to identify the stress tolerant genotypes.

CHAPTER 5

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

Soil acidity is one of the major constraints in the production of maize (Gichangi, 2007). Acidic soils not only reduce growth but also cause symptoms of nutrient deficiency, which significantly reduce maize yields (Narro *et al.*, 2001). Soil liming is one way of solving the problem of soil acidity but most farmers are poor and cannot afford this. Tailoring the crop to fit acidic soils is more effective and more economically and environmentally friendly than changing the soil to fit the crops.

Different screening methods were used in the current study in different environments (glasshouse, laboratory and field) to identify maize genotypes tolerant to low soil pH in the Eastern Cape province of South Africa. The general objective of the study was to determine the response of different varieties to acidic soils under laboratory, glasshouse and field conditions. The specific objectives were:

1) To identify maize genotypes that are tolerant to acidic soils.
2) To identify secondary traits that are associated with tolerance of maize genotypes to acidic soils.

The root growth stress tolerance index (RGSTI) was recorded in both the glasshouse and laboratory assays, and it consistently discriminated similar tolerant genotypes of maize, which were namely: Ngoyi, BG3492 BT, PAN 6Q408 and PHB 3442. The RGSTI proved to be the best index that was able to discriminate differences among genotypes under laboratory and glasshouse conditions.

Under field conditions, the top three performing genotypes at Mbinja were PHB33H56, PANBG3492 BT and PHB32W71, while at Mpumaze were PAN6P110, PAN6Q408 and PHB3442 based on HM and STI. The RGSTI was able to confirm two genotypes that were identified as tolerant by HM and STI under field conditions at Mpumaze village, namely PAN6Q408 and PHB3442.

It is apparent that two varieties (PAN6Q408 and PHB3442) were consistently tolerant when all the screening techniques are considered. The laboratory and glasshouse assays

screened the genotypes at early (seedling) stages of development, while field screening considered performance of the varieties till maturity. It is possible that different mechanisms of tolerance might be operating at seedling stage and during later stages of the plant's development.

The laboratory assay also focused on aluminium toxicity. It is known that there are two other possible toxicities in acidic soils, and these involve Fe and Mn (Tandzi *et al*., 2018). It is therefore possible that the other two toxicities might have been operative where soil was used, in the field and under glasshouse conditions. This might also explain the differential responses of the varieties that was observed.

The study revealed that plant height, leaf area and stem diameter could be used for indirect selection for tolerance to Al toxicity under glasshouse conditions. The seedling vigour index (SVI) was also very effective in identifying tolerant genotypes under glasshouse conditions. On the other hand, shoot length stress tolerance index and the haematoxylin score were useful for indirect selection for tolerance to Al toxicity in the laboratory.

In the field, it was revealed that ear length, leaf area and ear diameter can be useful in identifying genotypes that are tolerant to soil acidity. They can therefore be useful as indirect selection criteria under field conditions. Additionally, the best selection indices under field conditions were the Harmonic mean (HM) and the stress tolerance index (STI). The following recommendations can be made for future studies:

1) All varieties that were identified as tolerant are recommended for further evaluation in several soil acidity hot spots to confirm their tolerance and stability of performance under field conditions.
2) The tolerance mechanisms of these genotypes are not yet understood and thus require more research. Knowledge of the mechanisms of the tolerance of these maize genotypes could facilitate the development of more acid soil tolerant genotypes.

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